Exhibit 29

Pharmacodynamic and Pharmacokinetic Effects in Gingival Crevicular Fluid From Re-dosing During Brushing

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Abstract: *The IntelliClean System from Sonicare® and Crest® combines a rechargeable sonic power toothbrush and a novel liquid toothpaste into one integrated system, providing the opportunity to re-dose with toothpaste during the brushing cycle. The purpose of this study was to investigate cleaning effects from in-mouth re-dosing with toothpaste during the brushing cycle vs conventional bolus dosing. This was a randomized, examiner-blind, six-period, crossover clinical study. Eighteen adult subjects used an experimental integrated system employing either a re-dosing regimen (2 doses at the start of brushing with 1 additional in-mouth dose during the last 30 seconds of brushing [2+1]) or a conventional regimen (2 doses at the start of brushing only [2+0]). Gingival crevicular fluid (GCF) was sampled at the final brushing quadrant from a preselected site in the gingival sulcus using filter strips at baseline and at 4, 15, and 120 minutes postbrushing. Mean change from baseline in the concentrations of total facultative anaerobes (TFAs) and gram-negative anaerobes (GNAs) in the GCF at 120 minutes posttreatment were modeled separately using general linear mixed models. Area under the curve of surfactant (sodium dodecyl sulfate [SDS]) in GCF over 2 hours postbrushing was calculated and modeled using an analysis of variance model. All hypotheses were tested 2-sided at the 5% significance level. Relative to the conventional regimen, the re-dosing (2+1) regimen produced a significantly greater reduction in $\log_{10}$ (TFA colony-forming units [CFU]/μL GCF) after brushing, 0.99 ± 0.12 vs 0.65 ± 0.12 (mean change ± standard error), and a significantly greater reduction in $\log_{10}$ (GNA CFU/μL GCF) after brushing, 0.75 ± 0.14 vs 0.45 ± 0.14. The re-dosing regimen led to significantly more SDS in GCF relative to the conventional regimen over the 2-hour time period. Re-dosing of liquid toothpaste during the brushing cycle with the IntelliClean System leads to a significantly increased cleaning effect, as defined by a reduced bacterial count in GCF, and significantly higher levels of surfactant in the GCF up to 2 hours after the brushing event.*

Mechanical and/or chemotherapeutic cleaning of the oral cavity to remove or inhibit plaque growth remains the most effective means of promoting good oral health. Oral hygiene products continue to evolve rapidly by employing the latest advances in engineering, electronics, and chemistry to develop more effective cleaning technologies. Of those products available directly to the consumer, power toothbrushes have been recognized as making a positive contribution toward improved daily plaque control. Studies report improved plaque removal efficacy vs manual brushes as well as significant improvements in gingival and periodontal health.[1-4] Rechargeable power toothbrushes employing sonic technology are among the most innovative, delivering improved cleaning performance and an enhanced user experience.[5-7]

Similarly, toothpastes have improved as a result of advances in chemical formulation and the identification of novel ingredients. Stable fluoridated toothpastes containing either high-performance cleaning ingredients, substantive antibacterial agents, or combinations of the two are more readily formulated and can offer multiple oral health benefits.[8,9] A new system, the IntelliClean System from Sonicare[®a] and Crest[®b], has combined novel toothbrush and toothpaste technologies in an integrated oral hygiene product. The product uses a low-viscosity sodium-fluoride liquid toothpaste that has been specially developed to maximize both the cleaning performance of a sonic toothbrush and the in-use consumer experience. The stain, gingivitis, and plaque benefits of this system have been evaluated.[10-12] As well as enhanced cleaning, the system provides the opportunity to re-dose with toothpaste without interrupting the brushing cycle. Given the proven hydrodynamic action of sonic brush technology[7] and low-viscosity toothpaste, this unique feature may offer additional cleaning benefits.

Gingival crevicular fluid (GCF) is generally recognized as a key source of biomaterial suitable for studying the environmental factors associated with periodontal disease.[13,14] In clinically healthy individuals, GCF is a serum transudate, which can be readily collected from the sulcus after its passage through the junctional epithelium. As such, it contains most of the elements that are present in serum, but as a transudate into the gingival microenvironment it also is likely to contain plaque-derived microbes as well as related bacterial and host inflammatory response factors.[15] Quantitative and qualitative assessments of the GCF potentially can provide useful information about an individual's oral health status.[16] In certain situations, these evaluations may be particularly valuable in establishing cleaning or antibacterial performance of oral hygiene products.

Sodium dodecyl sulfate (SDS) is commonly used in personal cleansing and oral hygiene products, such as toothpaste. As a surfactant, it adds both esthetic and cleaning attributes to the formulation. Its chemical properties also may bestow limited bactericidal action, given the propensity of surfactants to break down phospholipid membranes.

The aim of this study was to determine the cleaning benefits from in-mouth re-dosing using a prototype integrated system representing the final and optimized brush design and toothpaste formulation.

Materials and Methods

Study Procedures

This was a randomized, single-center, examiner-blind, crossover clinical study. Eighteen adults (9 women and 9 men) with a mean age of 40 years were recruited. The study was limited to healthy individuals with gingival/periodontal pocket depths of 1 mm to 3 mm. All subjects gave written informed consent and agreed not to receive a dental prophylaxis or use any other dental product during the phase of active treatment. Subjects also were required not to use antibiotics during the study or within 2 weeks before the start of the study. At their initial visit, all subjects received a periodontal examination, including the identification of 3 gingival sites in the posterior upper quadrants that gave a crevicular fluid sample exceeding 25 Periotron[®c] units. Typically, mesiobuccal or mesiolingual sites were chosen and recorded for future use during the study. Pocket depth and bleeding on probing status were recorded for each GCF sampling site (Figure 1).

Subjects used the prototype integrated toothbrush/toothpaste system once during each treatment period under supervision according to their assigned usage/dosing instructions. Otherwise, subjects were supplied with Crest® Cavity Protection toothpaste[b] and Sonicare® Advance toothbrushes[a] to be used for the duration of the study.

Subjects received instructions to brush each of the 4 dental quadrants for 30 seconds. Half of the subjects brushed in the right-to-left order as follows: upper right (Q1), lower right (Q4), lower left (Q3), upper left (Q2) (Figure 2); the other half brushed in the left-to-right

[c]Oraflow, Inc, Plainview, NY 11803; 631-669-8954

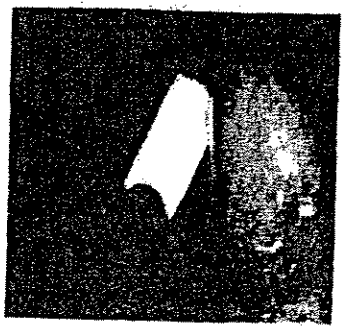


Figure 1—Sampling of gingival crevicular fluid.

[a]Philips Oral Healthcare, Inc, Snoqualmie, WA 98065; 800-676-SONIC
[b]The Procter & Gamble Co, Cincinnati, OH 45202; 800-492-7378

Table 1 Regimen Sequences

Regimen Sequence	Treatment Period 1	2	3	4	5	6
1	A	B	B	A	A	B
2	B	A	A	B	B	A
3	A	A	B	B	B	A
4	B	B	A	A	A	B

"A" and "B" represent the brushing regimens.

order as follows: upper left (Q2), lower left (Q3), lower right (Q4), upper right (Q1) (Figure 3).

After brushing, all subjects rinsed for 15 seconds with 30 mL of water. The treatment regimens employed a total of 2 minutes brushing time but differed with respect to the timing for the introduction of SDS-containing toothpaste into the mouth and the amount of SDS-containing toothpaste on the brush head, as follows:

Re-dose regimen (2+1): 2 pumps (~0.50 g toothpaste) delivered into the brush head to start brushing the upper quadrant (t = 0 seconds), 1 pump (~0.25 g toothpaste) delivered into the brush head to start brushing opposite the upper quadrant (t = 90 seconds). Re-dosing was reserved for the last 30 seconds of brushing, when the foam and body qualities of toothpaste are generally compromised from dilution effects.

Bolus-only regimen (2+0): 2 pumps (~0.50 g toothpaste) delivered into the brush head to start brushing the upper quadrant (t = 0 seconds).

Subjects were randomly assigned to one of the regimen sequences shown in Table 1 in a block size of 4 based on the subject's order of entry at the first day of treatment.

At the start of each treatment period, subjects had a baseline GCF sample taken from preidentified pharmacodynamic and pharmacokinetic sites. Subjects were blinded as to the location of these sites before study initiation. Subjects remained at the clinic for their 4- and 15-minute pharmacokinetic site GCF samplings and then returned to the clinic for pharmacokinetic and pharmacodynamic site samples 120 minutes after brushing. Subjects were not permitted to eat, drink, chew gum, or smoke between samplings.

Pharmacodynamic Procedures

1. Four Periotron® 8000 series instruments were calibrated before the start of the study according to the manufacturer's instructions. GCF samples were collected at the designated times by placing paper strips (PerioPaper®) at the entrance to the gingival sulcus for 30 seconds to absorb the fluid present.

2. GCF samples were collected at baseline from the upper quadrant (depending on the brushing sequence, the upper-left or the upper-right quadrant where brushing ended). Sampling sites were predetermined based on screening Periotron® values. A GCF sample was collected from the same sites used for baseline collection 120 minutes ± 5 minutes from the time the treatment was administered.

3. Periotron® units of the collected GCF volume were determined immediately after collection using the Periotron® 8000 series instruments. A calibration curve provided direct translation of Periotron® unit values to volumes that were reported in µL. The GCF volumes were used to obtain the final concentrations of bacteria expressed as colony-forming units

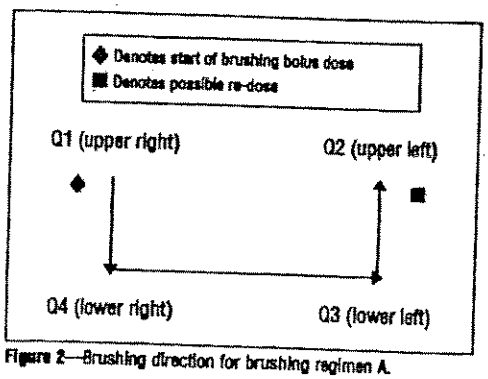


Figure 2—Brushing direction for brushing regimen A.

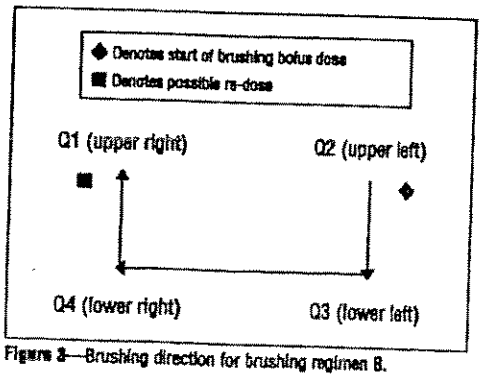


Figure 3—Brushing direction for brushing regimen B.

(CFU)/μL GCF.

4. Strips were cut with scissors to remove the orange plastic portion before placement into labeled sample vials containing 1 μL of Liquid Dental Transport (DTF)[4], caps closed, and then stored on ice before microbial processing. All pharmacodynamic processing was done within 2 hours of sample collection.

5. Appropriate cleaning and sterilization procedures were undertaken in between each sampling event.

6. To ensure mixing of the substrate before analysis, the Liquid Dental Transport was vortexed for 30 seconds at high speed before plating the sample. A neat sample (10^{-3}) and 1:10 (10^{-4}) and 1:100 (10^{-5}) dilutions of the neat sample were plated on Enriched Tryptic Soy Agar (ETSA)[4] for total facultative anaerobes (TFAs) and on Enriched Tryptic Soy Agar with nalidixic acid and vancomycin (ETSA-NV)[4] for gram-negative anaerobes (GNAs). All dilutions were prepared in sterile physiological saline. A spiral plater (Autoplater™ 4000[5]) was used for all platings.

7. Plates were incubated in an anaerobic chamber (AALC 3-DOOR[6]) at 37°C for 48 hours. Colonies were then enumerated using an automatic colony counter (QCount™[5]), and the counts were reported separately for the three dilutions as CFU/mL.

Oral hygiene products continue to evolve rapidly by employing the latest advances in engineering, electronics, and chemistry to develop more effective cleaning technologies.

Pharmacokinetic Procedures

1. Periotron® calibration, GCF sample collection, and quantification were performed as described for the pharmacodynamic procedures.

2. Strips were cut with scissors to remove the orange plastic portion before placement into a specified well of a 96-round, deep-well plate. Plate specification included subject identification, treatment period, time point, and whether the sample was collected from the upper-left or upper-right quadrant of the mouth. Plates containing the samples were stored frozen at –70°C until the time of analysis. Plates were removed from the freezer and allowed to reach room temperature before analysis.

3. Water (0.9 mL) was added to each well, followed by 0.1 mL of an internal standard solution containing a known mass of stable isotope labeled SDS (d_{25}).

Power toothbrushes have been recognized as making a positive contribution toward improved daily plaque control.

4. The plate was sealed with a cap mat and, to ensure mixing of the substrate before analysis, vortexed for 5 minutes before liquid chromatography tandem mass spectrometry analysis. An aliquot of the sample was injected onto an Xterra High Performance Liquid Chromatography column[4] using isocratic conditions. The column effluent was introduced into a triple quadrupole mass spectrometer (API 3000[b]) under turbo ion-spray conditions in the negative-ion–selected reaction monitoring mode. Transitions consisting of m/z 265 → 97 and m/z 290 → 98 were continuously monitored for SDS and d_{25}-SDS, respectively.

5. Calibration standards were prepared by spiking 1 μL of human plasma matrix containing a known mass of SDS onto PerioPaper® strips over the expected concentration range of the unknown samples. These standards were analyzed as described above. Samples with SDS concentrations exceeding the upper limit of the normal calibration range were further diluted with internal standard and reanalyzed.

6. A calibration curve was constructed by plotting peak area ratios of SDS/d_{25}-SDS vs the mass of SDS in each calibration standard. The mass of SDS contained in the unknown samples was then interpolated from the calibration curve. The mass of SDS in each unknown sample was then divided by the volume of GCF collected onto its respective strip to obtain the SDS concentration (ng SDS/μL GCF).

[4]Anaerobe Systems, Morgan Hill, CA 95037; 408-782-7557
[5]Spiral Biotech, Norwood, MA 02062; 800-554-1620
[6]COY Laboratory Products, Inc, Grass Lake, MI 49240; 734-475-2200
[a]Waters Corporation, Milford, MA 01757; 800-252-4752
[b]Applied Biosystems, Foster City, CA 94404; 800-327-3002

Table 2 Analysis of Covariance for Average Total Facultative and Gram-negative Anaerobes (TFA and GNA)

Regimen	Adjusted Mean Change	Adjusted Mean	Regimen Difference	Adjusted P-value
$\log_{10}$ (TFA CFU/μL GCF)				
2+1 regimen	−0.99 (0.12)	8.06 (0.12)	−0.34	.005
2+0 regimen	−0.65 (0.12)	8.40 (0.12)		
$\log_{10}$ (GNA CFU/μL GCF)				
2+1 regimen	−0.75 (0.14)	7.09 (0.14)	−0.30	.019
2+0 regimen	−0.45 (0.14)	7.38 (0.14)		

CFU = colony-forming units; GCF = gingival crevicular fluid.

Statistical Methods

For the pharmacodynamic measurements, the base-10 logarithm function was applied to the concentrations of GNA CFU/μL GCF and TFA CFU/μL GCF data before statistical analysis. For the pharmacokinetic measurement, the area under the SDS concentration curve (AUC) was calculated using the trapezoid rule for each subject. The SDS AUC was the primary measure of surfactant delivery to GCF. The analysis was performed on the natural logarithm scale of the original data. The adjusted means were transformed back to the original scale for the purposes of reporting.

The mean change from baseline in the number of GNA CFU/μL GCF and TFA CFU/μL GCF and SDS AUC were modeled separately using general linear mixed models. Each model included treatment and period as fixed class variables. Subject was included as a random class variable with compound symmetric within-subject correlation structure. The models of mean change from baseline bacteria concentration levels also included the respective baseline CFU/μL GCF ($\log_{10}$ scale) as a continuous covariate. These general mixed models were used to compare regimens at the 5% significance level.

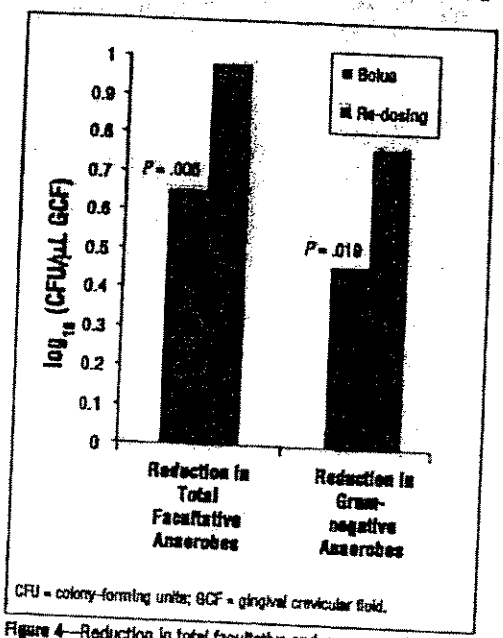


Figure 4—Reduction in total facultative and gram-negative anaerobic bacteria 120 minutes posttreatment.

Results

Study Population

All 18 subjects who were randomly assigned to 1 of the 4 regimen sequences completed all study visits. Subjects used each of the two treatment regimens three times during the course of the study. Subject sampling sites ranged from 2 mm to 3 mm probing depth, and 1 of 36 sample sites displayed bleeding when pocket depth was measured at the screening visit.

Pharmacodynamic Results

Before brushing, subjects had an average of 9.05 $\log_{10}$ TFA CFU/μL GCF and 7.84 $\log_{10}$ GNA CFU/μL GCF. After 2 hours postbrushing, the mean TFA reduction compared to baseline was significantly greater for the 2+1 regimen than for the 2+0 regimen (0.99 vs 0.65 $\log_{10}$ CFU/μL GCF, $P = .005$). There was also a significantly greater mean reduction of GNAs in GCF for the 2+1 regimen compared to the 2+0 regimen (0.75 vs 0.45 $\log_{10}$ CFU/μL GCF; $P = .019$) (Table 2 and Figure 4).

Pharmacokinetic Results

The mean SDS concentration in GCF was statistically significantly ($P < .002$) higher for the 2+1 regimen relative to the 2+0 regimen at each of the 4-, 15-, and 120-minute time points. Over the 2-hour postbrushing period, the SDS AUC was statistically significantly ($P < .0001$) greater for the 2+1 re-dosing regimen than for the 2+0 bolus-only regimen (29.5 vs 19.2 μg x min/μL) (Table 3 and Figure 5).

Regimen	Mean Area Under the SDS Concentration Curve (0 to 120 Minutes) in Gingival Crevicular Fluid (µg SDS × min/µL GCF)	Regimen Mean Value	Calculated P Value
2+1 regimen	29.6	1.54	< .0001
2+0 regimen	19.2		

SDS = sodium dodecyl sulfate.

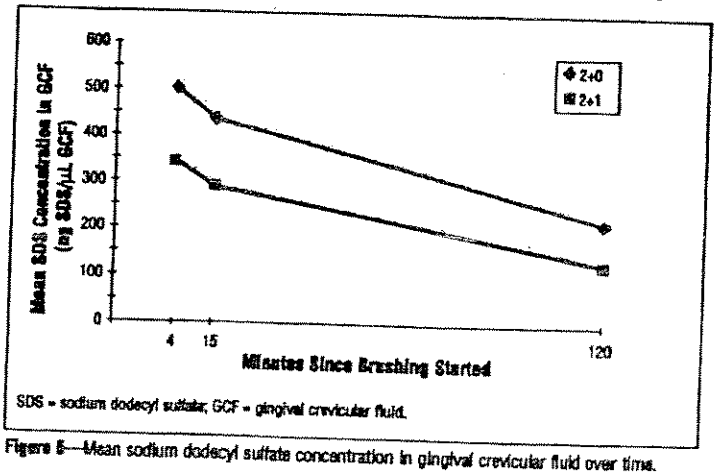
Discussion

The results of this study demonstrated the pharmacodynamic and pharmacokinetic benefits of re-dosing with toothpaste during the brushing cycle. A direct comparison of brushing cycles with and without an additional dose of liquid toothpaste indicated a significant reduction in TFAs and GNAs 2 hours after brushing had ceased. Re-dosing delivered approximately a log reduction in TFAs, a 52% greater effect than dosing only at the beginning of the brushing cycle. Differences for GNAs were similar, with a 66% greater log reduction in bacterial load in the GCF for the re-dosing vs regular regimen. The role of bacteria, specifically GNA classes, in the formation of plaque and subsequent initiation of gingivitis is well documented.[17-19] The ability of any oral hygiene regimen to positively affect the microbial flora within the sulcus is likely to aid the maintenance of healthy gingival tissues. The clinical relevance of such reductions remains to be established, although randomized clinical trials have already demonstrated antiplaque and antigingivitis effects for this integrated oral hygiene system.[10,11]

Similarly, the pharmacokinetic effects observed indicate the advantage of re-dosing to raise the amount of cleaning ingredients delivered into the gingival sulcus, thus prolonging the surfactant's activity in the oral cavity longer than with a bolus-only dose of toothpaste at the start of brushing. Over the 2-hour postbrushing period, the mean concentration of surfactant (SDS) in the GCF was 54% higher when an additional dose of toothpaste was dispensed during the brushing cycle compared to a bolus-only dose at the start of brushing. Importantly, the pharmacokinetic outcomes appear to be consistent with the pharmacodynamic results in supporting the overall cleaning benefits that can be observed in the GCF through re-dosing during the brushing cycle. From a pharmacokinetic perspective, there are clearly opportunities to explore the use of integrated antibacterial formulations and their impact on these evaluation parameters as a result of re-dosing.

The benefits of re-dosing during the brushing cycle are not yet fully characterized, and further experiments are required to fully understand the relative impact it may have vs the conventional toothbrush/toothpaste regimen. Research is ongoing to characterize the whole-mouth pharmacokinetic and pharmacodynamic profiles resulting from re-dosing if the actual quantity of product dispensed during the brushing cycle remains constant, ie, 3+0 vs 2+1. It is hypothesized that, by improving the distribution of the product within the mouth, the product's efficacy is less likely to suffer the dilution effects associated with bolus dosing. Supervised brushing to control brushing time and patterns was implemented to minimize individual habit influences and the impact, if any, of the subjects' awareness of sampling-site locations.

This research also focused on single-use effects, and it is clearly of interest to explore



SDS = sodium dodecyl sulfate; GCF = gingival crevicular fluid.

Figure 5—Mean sodium dodecyl sulfate concentration in gingival crevicular fluid over time.

the longer-term impact on cleaning using these evaluation parameters over time. Research reported elsewhere in this supplement indicates higher levels of compliance with the integrated system vs a separate toothbrush plus toothpaste over a 6-week period.[10] The result of that study indicated that user acceptance was higher with the integrated system, and it may be speculated that re-dosing plays an important role in this outcome by enhancing the usage experience.

Conclusion

Re-dosing with liquid toothpaste during the brushing cycle using an integrated sonic power toothbrush/low-viscosity liquid toothpaste leads to a significantly increased cleaning effect, defined as reduced bacteria count in GCF, vs bolus dosing at the start of brushing. Furthermore, re-dosing delivers significantly higher levels of surfactant to the GCF up to 2 hours after the brushing event vs bolus dosing at the beginning of the brushing cycle.

Disclosure

The Procter & Gamble Company supported this research.

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